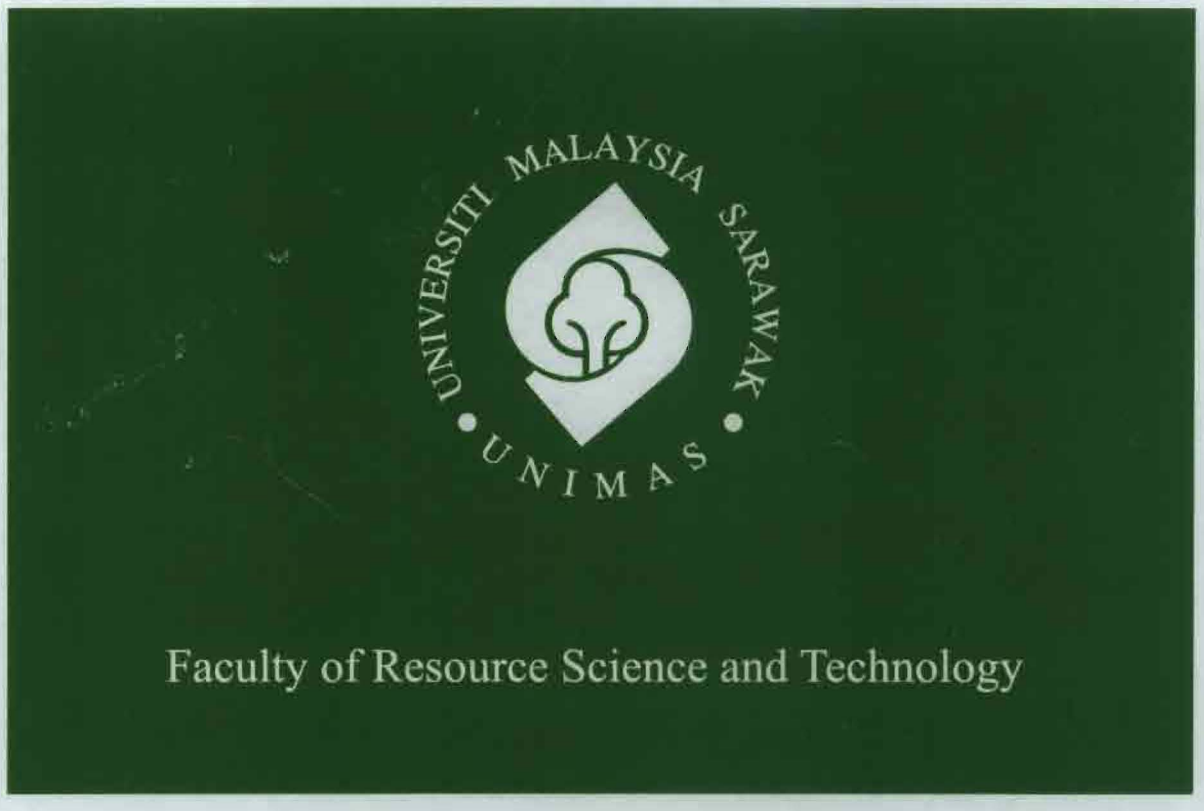


# GENE EXPRESSION ANALYSIS OF THE RIBOSOMAL PROTEIN GENE, *RPL 14* IN CELL LINES DERIVED FROM HUMAN NASOPHARYNGEAL EPITHELIUM.

**Lisha Vasudevan**

**(36771)**

**Bachelor of Science with Honours**
**(Resource Biotechnology)**
**2015**

**UNIVERSITI MALAYSIA SARAWAK**

Grade: _____

**Please tick (√)**

Final Year Project Report [√]

Masters [ ]

PhD [ ]

## DECLARATION OF ORIGINAL WORK

This declaration is made on the 02 day of June 2015 ~~2012~~.

**Student's Declaration:**

I Lisha Vasudevan, 36771, FSTS

(PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled Gene Expression Analysis of RPL14 in Nasopharyngeal Epithelium is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text, nor has any part been written for me by another person.

02/06/15
Date submitted

Lisha Vasudevan (36771)
Name of the student (Matric No.)

**Supervisor's Declaration:**

I Prof. Madya Dr. Edmund Sim Ui Hang (SUPERVISOR'S NAME) hereby certifies that the work entitled Gene Expression Analysis of RPL14 in Nasopharyngeal Epithelium (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment of Bachelor of Science with Resource Biotechnology (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work.

Received for examination by: Prof. Madya Dr. Edmund Sim Ui Hang
Jabatan Biologi Molekul
Fakulti Sains dan Teknologi Sumber
UNIVERSITI MALAYSIA SARAWAK
(Name of the supervisor)

Date: 08/06/15

I declare that Project/Thesis is classified as (Please tick (√)):

☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*
☑ **OPEN ACCESS**

**Validation of Project/Thesis**

I therefore duly affirm with free consent and willingly declare that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abiding interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitalise the content for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes the sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student signature _____ 02/06/15
(Date)

Supervisor signature: _____ 8/6/15
(Date)

Prof. Madya Dr. Edmund Sin Fui Hung
Jabatan Biologi Molekul
Fakulti Sains dan Teknologi Sumber
UNIVERSITI MALAYSIA SARAWAK

Current Address:

_____

_____

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument is duly prepared by The Centre for Academic Information Services]

## ACKNOWLEDGEMENT

Firstly, I would like thank the God who granted me all the grace and strength to complete this project to a success. I would also like to express my highest gratitude to my project supervisor, Associate Professor Dr. Edmund Sim Ui Hang who has been supervising and guiding me throughout the duration of my project as well as for his sharing his invaluable knowledge and his advice to conduct this final year project to a successful end. I hereby thank him for always being a support throughout the completion of my final year project.

Furthermore, I would like to use this opportunity to express my appreciation to all the postgraduate students of the Immunological Human Molecular Genetics Laboratory, UNIMAS. Paricularly, Ms. Stella Chan Li Li, Ms. Kherlee Ng, Ms. Felicia Kavitha Thomas and Ms. Shruti Talwar who have been consistently guiding me by sharing their invaluable experiences and generously helped and discussed my project even with their packed schedule in order to complete my final year project successfully. Moreover, I would also like to express my sincere appreciation to Ms. Limjatai Kadin Patrick who has been always kindly assissting me in the laboratory throughout the conduction of my laboratory work. In addition, I would like appreciate my lab mates, namely Najian Ibrahim, Yew Keh Li, Jaiyogesh Ramesh Patel and Cassandra Chee Sheau Mei, for their kindness, support and unity throughout my project to make it a success.

Last but not least, my genuine gratitude is for my beloved family, particularly my parents Mr. Vasudevan and Mrs. Devakee as well as my sibilings, Ms. Nisha and Mr. Karthikgheyan for their endless support and encouragement throughout the duration of my final year project. Their moral support and their trust on me have always been the bridge for the success of my project. In addition, I would like to thank all my friends and coursemates for supporting and encouraging me throughout the completion of my project.

## Table of Contents

## LIST OF ABBREAVIATION

| | |
|---|---|
| **AGE** | Agarose Gel Electrophoresis |
| **BLAST** | Basic Local Alignment Search Tool |
| **cDNA** | Complementary deoxyribonucleic acid |
| **DNA** | Deoxyribonucleic acid |
| **dNTP** | Deoxyribonucleotide triphosphate |
| **EBV** | Epstein–Barr Virus |
| **GAPDH** | Glyceraldehyde-3-phosphate dehydrogenase |
| **$MgCl_2$** | Magnesium chloride |
| **M-MLV** | Moloney Murine Leukaemia Virus |
| **mRNA** | Messenger RNA |
| **NPC** | Nasopharyngeal Carcinoma |
| **NSCLC** | Non-Small Cell Lung Cancer |
| **PCR** | Polymerase Chain Reaction |
| **RPs** | Ribosomal Proteins |
| **RPL14** | Ribosomal Protein Large 14 |
| **rpm** | Revolutions per minute |
| **rRNA** | Ribosomal RNA |
| **RT-PCR** | Reverse Transcriptase Polymerase Chain Reaction |
| **Tm** | Melting temperature |
| **µg** | Microgram |
| **µl** | Microliter |
| **µM** | Micromolar |
| **WHO** | World Health Organization |

## LIST OF TABLES

## LIST OF FIGURES

# Gene Expression Analysis of the Ribosomal Protein Genes, *RPL 14* in Cell Lines derived from Human Nasopharyngeal Epithelium.

**Lisha Vasudevan (36771)**

Resource Biotechnology
Department of Molecular Biology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

## ABSTRACT

Ribosome is an essential cellular organelle that is responsible in protein production. Many ribosomal proteins (RP) are involved in various roles besides protein biosynthesis, called extraribosomal functions. Ribosomal protein genes are associated with many genetic diseases and cancers. Previous studies have revealed difference in RP regulation of nasopharyngeal carcinoma (NPC) cell lines compared to the normal nasopharyngeal epithelium cell line. However, there has been no literature to date on the association between *RPL 14* and nasopharyngeal carcinoma. Hence, in this study relative expression pattern of *RPL 14* in NPC cell line, HK1, compared to the normal nasopharyngeal epithelium cell line, NP69 was observed using molecular technique such as Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). This study revealed specific PCR product with the estimated size of 773 bp for *RPL 14*. *RPL 14* did not show differential expression in NPC; but this does not suggest that it is not associated with nasopharyngeal carcinoma.

*Key word*: Extraribosomal function, nasopharyngeal carcinoma, RT-PCR, ribosomal proteins and *RPL 14*.

## ABSTRAK

*Ribosom telah dikaitkan dengan penghasilan protein. Protein ribosom mempunyai banyak keupayaan selain daripada penghasilan protein dikenali sebagai fungsi tambahan ribosom. Sesetengah gen protein ribosom dikaitkan dengan pelbagai penyakit seperti penyakit genetik dan kanser melalui ekspresi gen. Kajian sebelum ini membuktikan perbezaan mengenai pengawalan protein ribosom dalam karsinoma nasofarinks berbanding dengan garis sel normal epitelium nasofarinks .Walau bagaimanapun, tiada kajian dibuat antara RPL 14 dan karsinoma nasofarinks . Dalam kajian ini, corak pengekspresian RPL 14 telah dikaji dalam sel NPC, HK1 yang telah dibandingkan dengan pengekspresiannya dalam sel normal, NP69. Analisis transkrip telah dilakukan dengan menggunakan teknik "Reverse Transcriptase PCR" (RT-PCR). Kajian ini menghasilkan produk PCR dengan anggaran saiz 773 bp untuk RPL 14. RPL 14 didapati tidak diekspresi di sel NPC, tetapi ini tidak mencadangkan tiada hubungan antara gen ini dengan karsinoma nasofarinks.*

**Kata kunci**: *Fungsi Tambahan Ribosom, karsinoma nasofarinks, RT-PCR, protein ribosom dan RPL 14.*

## 1.0 INTRODUCTION

Gene expression is the most fundamental level at which proteins or RNA products are expressed and carry out its function through transcription and translation process. Ribosome is an essential cellular organelle used for protein synthesis in all cells which consists of ribosomal RNAs (rRNAs) and ribosomal proteins (RPs). RPs also has several importances that are independent of protein biosynthesis, called extraribosomal function (Mao-De & Jing, 2007).

Extraribosomal functions of human ribosomal proteins (RPs) include the regulation of cellular growth and differentiation, and are inferred from studies that relate hereditary disorders and cancer to the deregulated expression of RP genes. Studies by Sim *et al.* (2010) showed the extraribosomal roles in the context of human nasopharyngeal development, through the involvement of *RPL 27, RPL 37a* and *RPL 41* in nasopharyngeal carcinoma (NPC) tumourigenesis.

Although many molecular studies have been carried out, NPC remains one of the most commonly misdiagnosed diseases due to the nature of the disease itself (Cho, 2007). The development of a suitable biomarker is important and essential in the early diagnosis of the disease to better control the prognosis of the cancer. Thus, further studies on the characterization of expression pattern of *RPL 14* are necessary since this gene has not been studied before in NPC.

Decreased expression of *RPL 14* was observed in lung, oral and oesophagus carcinoma (Huang *et al.*, 2006; Shriver *et al.*, 1998). Furthermore, non-random allelic loss on the short arm of chromosome 3 is the most crucial genetic abnormality that is associated with the tumorigenesis of nasopharyngeal carcinoma (Chow *et al.*, 2004). The

location of the human ribosomal protein *RPL 14* is on the chromosome 3p21.3 (Huang *et al.*, 2006). Non-random losses at chromosome 3p21.3 region have commonly been observed in many human malignancies including lung, breast, kidney, cervical and nasopharyngeal cancers, indicating that one or more tumour suppressor gene within this region may be involved in this carcinoma (Liu *et al.*, 2003). However, *RPL 14* gene has not been proven to be associated with nasopharyngeal carcinoma before.

In this study, *RPL 14* gene was derived from normal nasopharyngeal epithelium cell line (NP69) and NPC cell line (HK1), and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) were carried out, followed by agarose gel electrophoresis (AGE) and the relative quantification of gene expression was validated by using unpaired student's t-test with a confidence interval of 95%. The focus of this research is to compare the expression patterns of *RPL 14* gene in normal cell line, NP69 and carcinoma cell line, HK1 of the nasopharyngeal epithelium in order to observe the expression pattern of *RPL 14* in NPC.

The objectives of this study are:

1. To observe the expression of the *RPL 14* in normal cell line, NP69 and NPC cell line, HK1.

2. To compare expression patterns of *RPL 14*.gene in NPC cell line, HK1 and the normal human cell line, NP69.

The study revealed the relative expression patterns of *RPL 14* gene in NPC cell line and normal cell line with the estimated PCR product size of 773 base pair.

# 2.0 LITERATURE REVIEW

## 2.1 Ribosomal Protein and *RPL 14*

Ribosome is an essential cellular organelle, which is responsible for protein synthesis that consists of ribosomal RNAs (rRNAs) and ribosomal proteins (RPs) (Lai & Xu, 2007). Cell growth and proliferation are allied with the protein synthesis by ribosome biogenesis which is also involved in the production of four rRNA and consist of nearly 80 ribosomal proteins (Jung *et al.*, 2013). Ribosome has two subunits, large (L, 60S) and small (S, 40S) subunits. RPs combine with rRNAs, directly or bind to each other and the accessory factors called proteins associated with ribosome (PAR) combine with two ribosomal subunits to regulate its synthesis and recycling, such as initiation factors (IFs), elongation factors (EFs)( Mao-De & Jing, 2007).

Many RPs also plays different roles besides protein biosynthesis, called extraribosomal functions. Wool (1996) defined extraribosomal functions for 29 RPs of human, *E. coli* and other species, including DNA replication, transcription, DNA repair, RNA splicing and modification, cell growth, proliferation, apoptosis and development regulation, cellular transformation and others. Disruptions in the protein synthesis process of RPs will consequently deregulate the growth of the cells and results in alteration of the cell cycle (Chan & Sim, 2013).

Ribosomal protein gene mutations or disturbance in their expression levels were identified in many inherited genetic diseases and cancers such as Diamond-Blackfan Anaemia Syndrome, Tuner Syndrome, Noonan Syndrome, Camurati-Engelmann Disease, Bardet-Biedl Syndrome (Yang & Liu, 2005). In colorectal carcinoma, the overexpression of *RPS 3* (Pogue-Geile *et al.*, 1991), *RPS 19* (Kondoh *et al.*, 1992) and *RPL 7a* (Wang *et*

*al.*, 2000) have been reported. A tumour metastasis-related gene, *RPL 23*, was found to induce high invasiveness of a human lung adenocarcinoma cell line (Liu *et al.*, 2007).

In nasopharyngeal carcinoma (NPC), two RPs gene, *RPS 27* and *RPS 26* have been identified to be down-regulated in NPC tumours compared to normal cell line (Sim *et al.*, 2008). Furthermore, study by Sim *et al.* (2010) proved that extraribosomal roles of *RPL 27*, *RPL 37a* and *RPL 41* in NPC tumourigenesis which showed significant down-regulation.

Ribosomal protein *L14*, commonly known as *RPL 14* gene encodes a ribosomal protein which is a component of the 60S subunit and belongs to L14E family of ribosomal proteins. The location of the protein is in the cytoplasm of the cell and maps at chromosome 3p21. This gene contains a trinucleotide (GCT) repeat tract whose length is highly polymorphic. Diseases associated with *RPL 14* are *Mucocutaneous Leishmaniasis* and *Congential Myasthenic Syndrome* (Shriver *et al.*, 1998). Figure 1 shows the location of human ribosomal protein gene *RPL 14* at chromosome 3 (3p21).

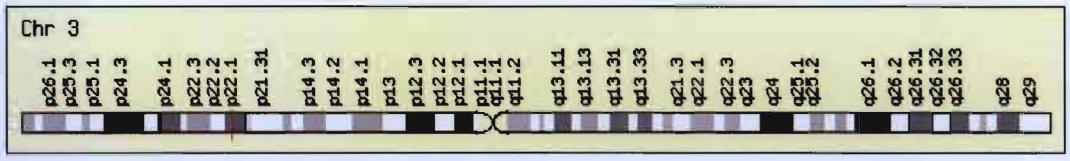


**Figure 1**: Location of *RPL 14* in chromosome 3 shown at the red line.

Adapted from http://www.genecards.org/cgi-bin/carddisp.pl?gene=RPL14.

## 2.2 Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a human squamous cell cancer which arises in the surface epithelium of the posterior nasopharynx tissue. NPC has its highest rate in Southern China and South East Asia, and is more widespread in the population of Cantonese-Chinese heritage (Tao & Chan, 2007). Interestingly, in the state of Sarawak, the native Bidayuh population was found to display highest age-standardized rates of NPC occurrence in the world (Devi *et al.*, 2004). Epstein–Barr Virus (EBV) infection is proposed as one of the significant etiological factors in NPC (Sham *et al.*, 1997). Figure 2 shows the location of nasopharyngeal tumour which is situated behind the nasal cavity above the soft palate.

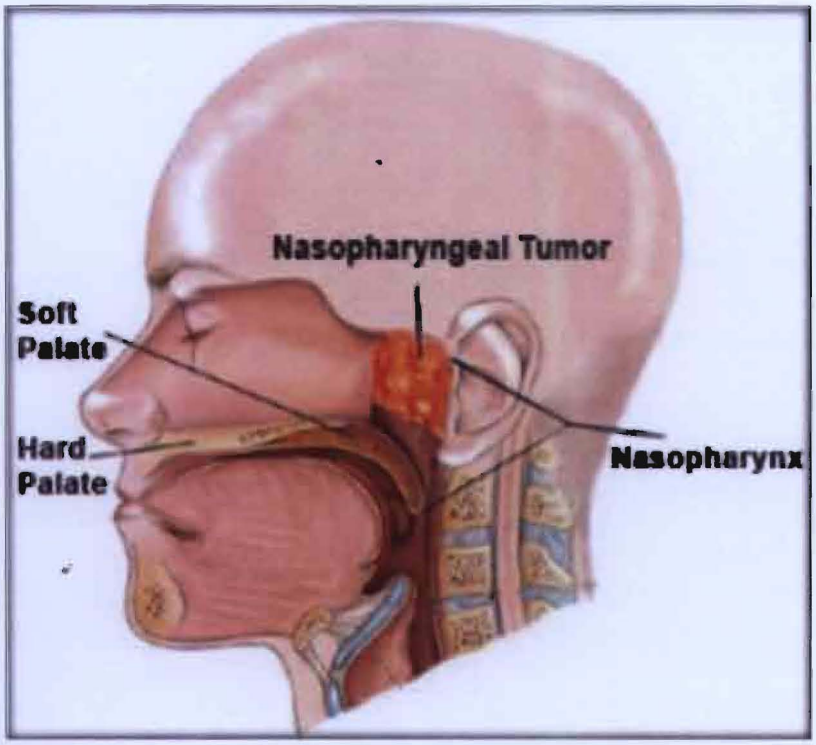


**Figure 2**: Location of Nasopharyngeal Tumour (Ramsay Sime Darby Health Care, 2013)
http://caringforcancer.areadigital.org/what-is-cancer/nasopharyngeal-cancer/

Nasopharyngeal Carcinoma is also known as epithelial neoplasm. According to the World Health Organization (WHO), NPC is classified into three histopathological types; Type 1 is the squamous cell carcinoma with distinct degrees of differentiation, Type 2 is non-keratinizing carcinoma and Type 3 is the undifferentiated carcinoma (Chan *et al.*, 2002).

Epithelial cell cancer generally arises from growth of squamous cell (80% of all nasopharyngeal tumours) at the lateral or posterosuperior walls of the nasopharynx (Carlos *et al.*, 1990). The occurrence of NPC is multifactorial in origin and multigenic in mechanism (Chien *et al.*, 2001). For instance, chromosomal deletion on the short arm of chromosome 3 is the most crucial genetic mutation that is associated with the tumorigenesis of nasopharyngeal carcinoma (Chow *et al.*, 2004).

## 2.3 Human Nasopharyngeal Cell Lines (HK 1& NP69)

Cell line has unique biological properties which will provide important information for the study of the tumorigenicity of a carcinoma. HK 1, a long term cell culture epithelial cell line which was established from a recurrent squamous carcinoma of the nasopharynx of 41 years old Chinese male 17½ years after radiation therapy. The tissue taken from the recurrent tumour after 17½ years was cultured in RPMI-1640 medium supplemented with 15% fetal calf serum to which penicillin and streptomycin were added. The cell line, HK 1 has been passaged 72 times *in vitro* over a period of approximately 1 year.

The cells have been shown by light and electron microscope to be the squamous epithelial type. Testing have been done through the transplantation into the back of athymic nude BALB/c (nu/nu) mice, tumours developed at the sites of inoculation, which on histological examination were observed to be well-differentiated squamous carcinomas. Karyotype analysis of cells from the HK 1 cell line shows an aneuploidy human type with

a modal chromosome number of 74 with both numerical and structural aberrations (Huang *et al.*, 1980).

This study also will be using NP69 cell line, which is non-tumorigenic and exhibit anchorage-dependent growth. This immortalized normal nasopharyngeal epithelium cell line acts as a control in this project. NP69 cell lines provide potential nasopharyngeal epithelial cell models for studying mechanisms involved in tumourigenesis of NPC. Primary cultures of nasopharyngeal epithelial cells are established from biopsies from the nasopharynx of patients with symptoms such as nasal obstruction and excessive bleeding. It is best cultured in culture medium RPMI1640 supplemented with fetal bovine serum (Tsao *et al.*, 2002).

## 2.4 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR is one of variations of polymerase chain reaction (PCR). This technique is mostly used to detect RNA expression in molecular biology (Freeman *et al.*, 1999). It allows the formation of the cDNA from RNA, which stores the sequence of RNA in the more stable form of nucleic acid, DNA.

RT-PCR is a semi-quantitative PCR and it can be carried out by the one-step RT-PCR protocol or the two step RT-PCR protocol. One step RT-PCR takes the mRNA targets and subjects them to reverse transcription and proceed to PCR amplification in a single test tube and it requires sequence- specific primer whereas two-step RT-PCR occurs in two steps as the name implies, step one is formation of cDNA from RNA and step two is the amplification process. The primer for two-step does not have to be sequence specific and this method is more sensitive than the one-step method (Schmittgen *et al.*, 2000).

RT-PCR is widely used in the diagnosis of genetic diseases and, semi-quantitatively, in the determination of the abundance of specific different RNA molecules within a cell or tissue as a measure of gene expression. RT-PCR can be used to diagnose genetic disease such as Lesch–Nyhan syndrome. This genetic disease is caused by a malfunction in the HPRT1 gene, which clinically leads to the fatal uric acid urinary stone and symptoms similar to gout (Joyce, 2002). Furthermore, RT-PCR is used in cancer detection to help improve prognosis, and monitor response to therapy. The goal is to determine which mRNA transcripts serve as the suitable biomarkers for a particular cancer cell type and then analyse its expression levels with RT-PCR. RT-PCR is also commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as Influenzavirus A and retroviruses like HIV (Xi *et al.*, 2007)

## 3.0 MATERIAL AND METHODS

Table 1 and Table 2 shows the list of materials and reagents as well as the tools and apparatus used in this study respectively.

**Table 1**: Materials and Reagents

| **Materials and Reagents** | **Brand** |
|---|---|
| dNTP Mix | Promega, UK |
| 10x Taq Buffer with KCl | Fermentas, USA |
| 25mM $MgCl_2$ | Promega, UK |
| Moloney Murine Leukaemia Virus Reverse Transcriptase | Promega, UK |
| Recombinant Taq DNA Polymerase | Fermentas, USA |
| TRIzol reagent | Invitrogen™, USA |
| Gene Ruler DNA Ladder Mix | Fermentas, USA |

**Table 2**: Tools and Apparatus

| **Tools and Apparatus** | **Brand** |
|---|---|
| E-Centrifuge | Hettich Zentrifugen, Germany |
| Express Cool Turbo Freezer (GR-T452ZV) | LG, Korea |
| NCBI primer designing tool | http://www.ncbi.nlm.nih.gov/ |
| Polymerase Chain Reaction (PCR) Machine | SensoQuest, Germany |
| Power Pack™ Power Supply 3000 | Major Science, USA |
| RNA Wood Hood | Köttermann, Germany |
| TotalLab Quant Software | TotalLab, USA |
| UV Spectrophotometer | Ultrospec 1100 pro, UK |
| UV Transluminator | Wise UV.WUV-M10, Korea |

## 3.1 Cell lines

Normal nasopharyngeal epithelial cell line NP69, and human NPC cell line HK1 were required for this research. The sources of the cell lines are presented in Table 3.

**Table 3**: Source of cell lines

| Designated Name | Type of cell line | Source |
|---|---|---|
| **NP69** | Normal Human Nasopharyngeal Epithelial | Prof. Tsao Sai Wah from Department of Anatomy, University of Hong Kong |
| **HK1** | Human NPC | Faculty of Health and Medical Science, Universiti Malaysia Sarawak |

## 3.2 Primer Synthesis

Forward and reverse primers for *RPL 14* transcript variant 1 (Accession: NM_001034996, 939 base pair) and *RPL 14* transcript variant 2 (Accession: NM_003973, 875 base pair) was used in this research. An internal control gene Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) transcript variant 1(Accession: NM_002046, 1421 base pair), *GAPDH* transcript variant 2 (Accession: NM_001256799, 1455 base pair), *GAPDH* transcript variant 3 (Accession: NM_001289745, 1513 base pair) and *GAPDH* transcript variant 4 (Accession: NM_001289746, 1407 base pair) was used as a reference gene for the RT-PCR to normalize the band intensity of the cell lines. The parameters of the primers were measured using Oligonucleotide Properties Calculator and OligoAnalyzer 3.1 software.

### 3.3 Extraction of Total RNA

TRIzol method (Chomczynski, 1993) was used to isolate total RNA from the normal human nasopharyngeal cell line and NPC cell line separately.

The culture media was discarded from the culture flask and the cell monolayer was rinsed with 1 mL of ice cold PBS twice. 1 mL of TRIzol reagent per 3.5 cm diameter dish was added and the cell monolayer was then scrapped using a cell scraper. Then it was incubated for 5 minutes. The cell lysate must be passed several times through a pipette before transferring the cell lysate into a new microcentrifuge tube. 0.2 mL of chloroform was added per 1 mL of TRIzol reagent and the samples were vortexed vigorously for 15 seconds and incubated at room temperature for 5 minutes. Later, the samples were centrifuged at 10000 rpm for 10 minutes at 4 °C.

Upon centrifugation, the mixture was separated into three layers, where only the upper clear aqueous layer containing RNA was transferred without disturbing the interphase into a clean 1.5 ml tube. 0.5 mL of isopropyl alcohol per 1 mL of TRIzol reagent was added and mixed by inverting the tube. The samples were then centrifuged at 10000 rpm for 5 minutes at 4 °C in order to pellet the RNA and the supernatant was discarded completely. 1 mL of 75% molecular grade ethanol was added to wash the RNA pellet. The ethanol was discarded and the pellet was completely air-dried for 5-10 minutes. Finally, the RNA was dissolved in 30 µl of nuclease-free water by pipetting.

### 3.4 RNA Quantification

Following the RNA extraction, agarose gel electrophoresis (AGE) was conducted in order to check the quality of the RNA. 1% agarose were used. AGE was conducted at 100V for 50 minutes. Concentration and purity of RNA isolated was determined by measuring absorbance via UV spectrophotometer at 260 nm and 260/280 ratio respectively (Shimadzu, USA). Volume of 1µL of RNA from each cell lines which were NP69 and HK1 were diluted separately in 49 µL of nuclease free water to make 50X dilution factor in a glass cuvette. The prepared RNA sample was inserted into the UV spectrophotometer to read the absorbance values. The readings were then recorded.

### 3.5 cDNA Synthesis

cDNAs was synthesized using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV) (Promega, UK).

According to the Promega protocol, 2 µg of RNA was added with 1 µL of random primers top up with nuclease free water up to a total volume of 15 µL. The RNA mixture was then heated at 70 °C for 5 minutes to melt the secondary structure within the template and cooled immediately on ice to prevent secondary structure from reforming. The tube was spinned briefly to obtain the bottom layer. 5 µL of M-MLV 5X reaction buffer, 1.25 µL of dNTP, 0.625 µL of Recombinant RNasin® Ribonuclease Inhibitor, 1 µL of M-MLV RT and a total volume 25 µL of nuclease free water was added. The samples must be mixed gently by flicking the tubes which were then incubated for 60 minutes at 37 °C. The synthesized cDNA were analysed on Agarose Gel Electrophoresis. The synthesized cDNA of NP69 and HK1 were stored at -20°C until they were used for the downstream procedure, which is the exponential amplification of the cDNA by DNA polymerase.

### 3.6 Polymerase Chain Reaction (PCR)

Taq Polymerase Chain Reaction protocol adopted from Fermentas (USA) was used. First, the following components shown in Table 4 were added into a sterile microcentrifuge tube on ice.

**Table 4**: PCR Mix (Fermentas, USA).

| Components | Final Volume | Final Concentration |
|---|---|---|
| 10 X Taq Buffer | 2.5µl | 1X |
| $MgCl_2$ solution, 25Mm | 1µl | 1.0mM |
| dNTP Mix, 10Mm | 0.5µl | 0.2 mM |
| Forward primer | 2.5µl | 1.0µM |
| Reverse primer | 2.5µl | 1.0µM |
| Taq DNA polymerase | 0.125µl | 1.25u |
| Template DNA | 1µl | <0.5µg/25µl |
| Top up Nuclease free water to | 25µl | |

The PCR was carried out for each gene in two different cell lines which were NP69 and HK1 with the parameters below, where the annealing temperatures vary accordingly for the gene *RPL 14* and the control housekeeping gene *GAPDH* which was represented as *x*. Table 5 shows the thermal cycling conditions for PCR.

**Table 5:** Thermal Cycling Conditions for PCR (Fermentas, USA).

| Step | Temperature | Time | Number of cycles |
|---|---|---|---|
| Initial denaturation | 95°C | 2 minutes | 1 cycle |
| Denaturation | 95°C | 30 seconds | |
| Annealing | *x*°C | 30 seconds | 30 cycles |
| Extension | 72°C | 1 minutes | |
| Final Extension | 72°C | 5 minutes | 1 cycle |
| Soak | 4°C | ∞ | 1 cycle |

### 3.7 Agarose Gel Electrophoresis and Sequencing

1% agarose gel was prepared and the PCR products were visualized with ethidium bromide. AGE was conducted at 100V for 50 minutes. Gel image was observed using an UV transilluminator. The PCR products were sent to the First Base Company for sequencing. The sequencing data obtained was analyzed and gene identification was done with BLAST search to verify the similarities between the sequence obtained with sequence in the GenBank.

### 3.8 Data Analysis

The band intensity was obtained from the AGE and incorporation of software called TotalLab Quant (TotalLab, USA) was used to analyze the differential expression patterns of *RPL 14* and *GAPDH*. The ratio of the band intensity values were calculated by using the following formulas:

$$Fold\ difference\ of\ target\ gene = \frac{Band\ intensity\ of\ target\ gene}{Average\ band\ intensity\ of\ reference\ gene}$$

$$Fold\ difference\ of\ gene\ in\ NPC = \frac{Expression\ of\ target\ gene\ in\ NPC}{Expression\ of\ target\ gene\ in\ normal\ cell\ line}$$

Relative quantification of gene expression was validated by using unpaired student's-test with a confidence interval of 95%, in which $p$-value smaller than 0.05 was needed to reject the null hypothesis which represents that there is significant difference in the expression patterns of the genes in normal cell line and NPC cell lines.